far as setting the penetrance for the disease allele in the kindred, we maintain that the disease allele is highly penetrant in this family. Until the disease gene is identified and all individuals are genotyped, it is not possible to estimate the penetrance more accurately. The concern regarding the definition of a distal flanking marker on the basis of recombination in an unaffected individual is legitimate. On the basis of our clinical assessment, however, we think it highly unlikely that the individual in which the recombination event occurred is a disease-allele carrier.

Note added in proof.—A gene for HSAN I was recently assigned to the 9q22.1-q22.3 region (Nicholson et al. 1996). Although we have not tested for linkage with the 9q markers in the family that we classified as having HMSN II and for which we mapped the disease locus to 3q, it is highly unlikely that the disease gene in our family is linked to 9q22.2-q22.3 sequences. The assignment of HSAN I to 9q adds further weight to our argument that the family we investigated has HMSN II and not HSAN I, as suggested by Vance et al.

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Elevated Total Plasma Homocysteine and 677C→T Mutation of the 5,10-Methylenetetrahydrofolate Reductase Gene in Thrombotic Vascular Disease

To the Editor:

Moderate elevation of total plasma homocysteine (tHcy) has been reported as an independent risk factor for thrombotic vascular disease, a well-known multifactorial disorder. Possible genetic causes of elevated tHcy include defects of the sulfur-containing amino acids metabolism due to deficiencies of cystathionine β-synthase, of 5,10-methylenetetrahydrofolate reductase (MTHFR), and of the enzymes of cobalamin metabolism. An impaired activity of MTHFR due to a thermolabile form of the enzyme has been observed in ≤28% of hyperhomocysteinemic patients with premature vascular disease (Engbersen et al. 1995). More recently, the molecular basis of such enzymatic thermolability has been related to a common mutation of the MTHFR gene, causing a C-to-T substitution at nt 677 (677C→T) (Frosst et al. 1995). This mutation was found in 38% of unselected chromosomes from 57 French Canadian individuals. The homozygous state for the mutation was present in 12% of these subjects and correlated with significantly elevated tHcy. Preliminary evidence indicates that the frequency of homozygotes for the 677C→T mutation may vary significantly in populations from different geographic areas (from 1.4% to 15%; Motulsky 1996).

We evaluated the frequency of the 677C→T mutation of the MTHFR gene in an Italian population of 64 unrelated patients with early-onset venous or arterial occlusive disease or with thrombosis occurring at unusual sites who were referred to a thrombosis research unit of

a community hospital (table 1). In 29 patients (16 men and 13 women, mean age 42.6 ± 11.8 years, mean age at event 36.7 ± 11.4 years), fasting tHcy—on at least two separate samples—was above the 95th percentile of the distribution in age-matched control men (19.5 μM) and women (15 μM) (Fermo et al. 1995) (mean tHcy 28.6 ± 15.7 μM). Seven of them presented arterial thrombotic events (stroke, transient ischemic attacks), while 22 had venous occlusive disease (deep vein thrombosis, pulmonary embolism, cerebral vein thrombosis, central retinal vein occlusion). This group of 29 patients was free from risk factors known to disturb methionine metabolism (diabetes, hypertension, hyperlipidemia and renal failure) and had normal plasma levels of the cofactors vitamin B_{12} (344 ± 107 ng/ml; lower normal limit 200 ng/ml) and folate (5.5 ± 2.2 ng/ml; lower normal limit 3 ng/ml). The remaining 35 patients (18 men and 17 women) were comparable in terms of age (41.3 ± 14.1 years), age at first event (36.2 ± 11.9 years), and type of event (7 with arterial and 28 with venous occlusive events), but they had normal fasting tHcy (13.0 ± 3.1 μM). Two-hundred fifty-eight apparently healthy subjects free of vascular disease served as controls. Analysis of the 677C→T mutation of the MTHFR gene was carried out as described by Frosst et al. (1995).

Table 1

Demographic Characteristics of the Patient Population

	Patients	
	With Elevated tHcy	With Normal tHcy
Arterial occlusive disease:		
Male/Female (No.)	3/4	5/2
Age (years)	40.9 ± 8.7	48.3 ± 10.3
Age at event (years)	36.3 ± 8.2	39.8 ± 12.2
tHcy (μM)	27.2 ± 12.1	15.2 ± 2.3
Ischemic stroke (No.)	6 (4)[a]	3
Central retinal artery occlusion (No.)	...	2
Transient ischemic attacks (No.)	1 (1)	1
Myocardial infarction (No.)	...	1
Venous occlusive disease:		
Male/Female (No.)	13/9	13/15
Age (years)	43.1 ± 12.7	39.5 ± 14.6
Age at event (years)	36.9 ± 12.4	35.3 ± 12
tHcy (μM)	29.5 ± 16.7	12.4 ± 3.1
Pulmonary embolism (No.)	2 (1)	1
Deep vein thrombosis (No.)	14 (8)	17
Cerebral vein thrombosis (No.)	2	
Central retinal vein occlusion (No.)	1 (1)	7 (1)
Superficial vein thrombosis (No.)	3 (3)	1

NOTE.—Among patients with elevated tHcy the prevalence of the +/+ genotype was not different in arterial (71%, 95% confidence interval 30%–95%) and venous patients (59%, 37%–78%).

[a] The number of patients with the +/+ genotype is shown in parentheses.

Table 2

Prevalence of Genotypes Identified by the 677C→T Mutation in Cases and Controls

	Genotype[a] (%)		
	−/−	+/−	+/+
Patients with elevated tHcy	10.3 (3)	27.6 (8)	62.1 (18)
(n = 29)	2.7–28.4	13.4–47.5	42.3–78.7
Patients with normal tHcy	28.6 (10)	68.6 (24)	2.8 (1)
(n = 35)	15.3–46.5	50.6–82.6	0–16.7
Controls (n = 258)	34.9 (90)	50.0 (129)	15.1 (39)
	29.2–41.1	43.8–56.2	11.1–20.2

NOTE.—Patients with elevated tHcy vs. patients with normal tHcy $\chi^2 = 27.3$; $P < .0001$.

[a] Range is 95% confidence interval; number of patients given in parentheses.

Homozygosity for the 677C→T mutation (+/+) was found in 15.1% (95%, confidence interval 11.1%–20.2%) of the individuals in the control population (table 2), a prevalence significantly higher than that reported by Kluijtmans et al. (1996) in the Dutch general population (5.4%; 95% confidence interval 2.2%–11.9%). Among the 35 thrombophilic patients with normal tHcy, only 1 was homozygous for the mutation. In contrast, the +/+ genotype was observed in 18 of the 29 patients with elevated tHcy (9 men and 9 women, 5 with arterial and 13 with venous thrombotic events). This figure is significantly higher ($P = .01$, χ^2 test) when compared to the frequency of MTHFR thermolability (28%) observed by Engbersen et al. (1995) in Dutch patients with elevated tHcy and premature vascular diseases. Total Hcy was not significantly different in the 18 patients homozygous for the mutation (32 ± 19 μM) and in the remaining 11 patients with elevated tHcy (22.9 ± 5 μM; $P > .15$, Mann-Whitney rank test).

These results confirm the variable frequency of homozygotes for the 677C→T mutation in different geographic areas and indicate that in an Italian population of patients with premature venous or arterial thrombotic diseases homozygosity for the 677C→T of the MTHFR gene is the prevalent cause of elevations in fasting tHcy. Nevertheless, other factors—either genetic or environmental—are responsible for elevated tHcy in a substantial proportion of patients with vascular occlusive diseases.

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Evidence for Genetic Anticipation in Non-Mendelian Diseases

To the Editor:

Genetic anticipation (GA) is characterized by a reduction in the age at disease onset (AAO) and also by an increase in both the severity and proportion of affected individuals in successive generations. Mott was the first to coin the term "anticipation," in 1911 (Mott 1911). For almost 80 years the finding of GA was attributed to ascertainment biases. Although such biases may exist, it is difficult either to confirm their presence or to exclude them. This controversy was clarified, for a number of rare Mendelian disorders, by the discovery of unstable DNA. Unstable DNA consists of multiple trinucleotide repeats that tend to increase in subsequent generations (Mandel 1994). The ability of unstable triplets to increase in number across generations provided a molecular basis for GA, and concern about ascertainment biases diminished.

We noticed a reduction of AAO in younger generations, for familial breast cancer (BRCA), colon cancer (COCA), Alzheimer disease (AD), maturity-onset diabetes of the young (MODY), and insulin-dependent diabetes mellitus (IDDM). These disorders differ from unstable DNA diseases because they belong to the category of common non-Mendelian disorders, with most cases occurring sporadically. In addition, disease genes with traditional DNA mutations (substitutions, deletions, and insertions) have been described for BRCA, COCA, and AD, with no evidence of trinucleotide expansion.

In order to verify this observation statistically, we collected the AAO for these non-Mendelian diseases (age at diagnosis for cancers) from available published pedigrees. On the basis of previous anticipation studies (McInnis et al. 1993), we chose two sampling strategies. First, direct transmission of disease in only parent-child pairs was analyzed, and the AAO in the parental generation (G1) was compared with that in the child's generation (G2); (this corresponds to "scheme 4," described elsewhere [McInnis et al. 1993]). In addition, for one BRCA study (Hall et al. 1990), we analyzed the data with all possible transmitting pairs—that is, each affected member of G1 was matched to each affected member of G2 ("scheme 3" [McInnis et al. 1993]). Scheme 4 was adopted for all the other analyses because the same highly statistically significant result was obtained for both sampling strategies. For some diseases the distribution of AAO was not normal; thus a Wilcoxon matched-pair analysis was used in addition to a paired *t*-test.

For BRCA, only females were included, and in cases of bilateral pathology the AAO of the earliest tumor was used. Initial analysis of directly transmitting mother-daughter pairs from a large BRCA linkage study ("BRCA Hall" [Hall et al. 1990]) was followed by analysis of all other recent available BRCA pedigrees together ("BRCA others" [Zuppan et al. 1991; Arason et al. 1993; Bowcock et al. 1993; Chamberlain et al. 1993; Cohen et al. 1993; Deville et al. 1993; Feunteun et al. 1993; Kelsell et al. 1993; Lindblom et al. 1993a; Mazoyer et al. 1993; Smith et al. 1993; Spurr et al. 1993;